UNIVERSITY OF ILLINOIS
DEPARTMENT OF BACTERIOLOGY
362 NOYES LABORATORY OF CHEMISTRY
URBANA

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Professor J. Lederberg
Department of Genetics
University of Wisconsin
Madison, Wisconsin

Dear Josh:

Here is a description of the shocking procedure which I have been promising to send on to you. We make the protoplasts the same way that you do. They are then spun down and subjected to one of two procedures: (a) The pellet is stirred to make subsequent resuspension easier. A volume of distilled water equivalent to 1/8 of the original volume is added to the pellet. A cork is inserted in the tube and shaken vigorously for uniform suspension. One then adds concentrated sucrose to yield a final concentration of 10%. The material is then spun and the pellet collected. (b) The protoplasts are spun and resuspended in 18% sucrose (or 0.5M Na Succinate), 1% casein hydrolysate, 0.6% HDP, 0.1M K Cl, 10^{-3}M Mg Cl_2, 10^{-4}M Mn Cl_2. The volume used should yield an eight-fold concentration of the protoplasm. Ten volumes of distilled water are added suddenly and within a minute concentrated sucrose added to yield a final concentration of 10%. The material is spun and the pellet collected. The initial suspending medium is also an induction medium when further supplemented with 1000 units of penicillin and 5 x 10^{-4}M TMG. The medium should have a pH of 6.9.

You will note that we can use either sucrose or succinate. This has turned out to be true both for shocking and enzyme induction. For the synthesis of RNA, succinate seems to be superior in the coli shockates, but this is really still under investigation.

Sincerely yours,

Sol

S. Spiegelman
Professor of Bacteriology

SS:wb

P.S. We are learning something new everyday and it is likely our procedures will be modified as we go along. If some striking improvement emerge I will let you know. We now have DNA and RNA synthesis in coli shockate, between 2 and 3x increases; as yet they are not as good as megaterium.

Spiegelman, S.